ONLINE METHODS

Microbial ligands and cytokines. For activation via NOD2 and TLR2/1, muramyl dipeptide (MDP, Invivogen) and the mycobacterial 19-kDa lipopeptide (EMC Microcollections)[24] were used at 1 μg ml^{-1} each or as indicated. For the differentiation of monocytes with cytokines, IL-32 (50 ng ml^{-1}, R&D Systems) and GM-CSF (1 U ml^{-1}, 1 U = 180 pg, Immunex) were used. These reagents were all tested for endotoxin by LAL Assay (Limulus amoebocyte lysate, Lonza) to be endotoxin free (detection limit <0.1 EU ml^{-1}). Other NLR and TLR ligands used were NOD1L (iE-DAP, 10 mg ml^{-1}), TLR2/6L (mycoplasma macrophage-activating lipopeptide-2 kDa, 1 μg ml^{-1}), TLR4L (lipopolysaccharide, 10 ng ml^{-1}), TLR5L (flagellum, 1 μg ml^{-1}), TLR7L (imiquimod, 5 μg ml^{-1}), TLR8L (single-stranded RNA, 0.5 μg ml^{-1}), *M. leprae* sonicate (10 μg ml^{-1}), *M. leprae* cell wall (10 μg ml^{-1}).

Monocyte isolation, DC differentiation and enrichment. We obtained whole blood from healthy donors (UCLA Institutional Review Board 92-10-591-31) with informed consent. PBMCs were isolated using Ficoll (GE Healthcare) gradient centrifugation, and monocytes were further enriched using Percoll density gradient (GE Healthcare) and subsequent adherence in 1% FCS for 2h. Monocyte purity was found to be >80%, as measured by CD14 expression. Cells were cultured for 48 h in RPMI and 10% serum, either FCS (Omega Scientific) or human serum. To purify CD1b$^+$ DCs, cells were cultured with the NOD2L, TLR2/1L, recombinant GM-CSF or rIL-32 for 48 h in RPMI with 10% FCS (Omega Scientific), labeled with a CD1b-specific antibody (Bcd3.1, American Type Culture Collection) followed by a microbead-coupled IgG1-specific secondary antibody (Miltenyi Biotec, 130-047-101, 1:5). The positive population was collected using magnetic-activated cell sorting (MACS) according to the manufacturer's recommendations (Miltenyi Biotec). Purity was confirmed to be generally >90% by flow cytometry.

Patients and clinical specimens. All patients with leprosy were recruited with informed consent and approval from the Institutional Review Board of the University of Southern California School of Medicine and the Institutional Ethics Committee of Oswald Cruz Foundation. Patients with leprosy were classified according to the criteria of Ridley and Jopling[53]; all patients with T-lep were classified as borderline tuberculoid, and all patients with L-lep had lepromatous leprosy. All T-lep and L-lep biopsy specimens were taken at the time of diagnosis before treatment.

Gene expression profiles. RNA was isolated using Trizol reagent (Invitrogen) and further purified over RNAeasy mini kit columns (Qiagen). Probes were prepared according to the Affymetrix protocol by the UCLA Microarray Core Facility that performed the hybridization of the Affymetrix Human U133 Plus 2.0 array (Affymetrix). Monocytes were isolated from five healthy donors, cultured in RPMI with 10% vitamin D–sufficient (100 nM) human serum and microbial ligands. Medium, NOD2L- and TLR2/1L-stimulated samples were collected at 0 h, 6 h and 24 h. Gene expression analyses were performed as previously described[36] and compared to the gene expression profiles from skin biopsy specimens from a total of 17 patients with leprosy (T-lep, $n = 10$; L-lep, $n = 7$) as previously reported[36]. The acquisition and initial quantification of array images were conducted using the AGCC software (UCLA core, Affymetrix). The subsequent data analysis was performed using Partek v6.4, and further biofunctional analysis was performed using IPA Software. Each gene was ranked by the probability that the means of its expression values are statistically distinct between medium and NOD2L- or TLR2/1L-treated samples using the Student's t test. We focused on genes meeting the criteria $P < 0.05$ and fold change >1.5. Enrichment analysis results were corrected for multiple hypothesis testing using the Benjamini-Hochberg method to control for false discovery. Statistical analysis of differential expression patterns for the group I CD1 antigen presentation molecules were done using the least differentially expressed gene as an upper bound on the significance P value.

Cell surface labeling and ELISA. Cell surface expression of antigenic determinants was determined using epitope-specific antibodies, and cells were acquired and analyzed as described[10]. Fluorochrome-coupled monoclonal antibodies to the following proteins were used: CD40 (BD, 555588, 1:10), HLA-ABC (BD, 555553, 1:10), HLA-DR (BD, 555560, 1:10), CD80 (BD, 557226, 1:10), CD86 (BD, 555658, 1:10) and CD209 (BD, Clone DCN46, 1:10)). For detection of CD1, a monoclonal primary antibody (Bcd3.1, American Type Culture Collection) was used, followed by an IgG1-specific secondary antibody (Invitrogen, A10541, 1:50). Secreted IL-32 protein in the supernatant was measured using an IL-32 Sandwich ELISA kit (SEL101, YbdYbiotech, Korea).

T cell assays. For investigation of MHC class II–restricted antigen presentation to T cells, monocytes were differentiated into immature DCs and enriched for CD1b$^+$ cells (as described above). Purified DCs were cultured with the MHC class II–restricted T cell clone derived from a patient with T-lep (1×10^5, BCD4.9) that recognized the *M. leprae* GroES protein and a defined peptide spanning amino acids 28–39 in an HLA-DR15–restricted manner[25]. IFN-γ was measured by ELISA (BD Pharmingen), and proliferation was measured using ^{3}H-thymidine incorporation as described[37]. For MHC class I–restricted antigen presentation studies, CD1b$^+$ DCs were prepared as above from tetanus toxoid–immunized healthy donors, under informed consent and approval from the UCLA IRB and the potential to present tetanus toxoid antigen (10 μg ml^{-1}) to autologous CD8$^+$ T cells was assessed (see above). The response of auto CD8$^+$ T cells to influenza peptide M158-66 (10 μg ml^{-1}, Princeton Biomolecules) was tested. In some experiments, the efficiency of antigen presentation by DCs was evaluated by performing titrations, varying the number of antigen-presenting cells, using an optimal concentration of antigen. The control without antigen performed at the highest concentration of CD1b$^+$ DCs is shown for each experiment (**Supplementary Table 5**). The mean values from proliferation assays and IFN-γ ELISA were always more than sevenfold greater in cultures with antigen versus medium control.

Real-time quantitative PCR. Following stimulation, RNA was isolated using Trizol (Invitrogen); cDNA and quantitative PCR performed as previously described[54]. Quantitect primers (Qiagen) were used. The relative quantities of the gene tested per sample were calculated against 36B4 using the Δ cycle threshold formula as previously described (isolated RNA and synthesized cDNA as described)[55]. The data were normalized by fold change to medium control samples.

Immunoperoxidase and immunofluorescence. Immunoperoxidase and double immunofluorescence labeling on leprosy skin lesions was performed and examined as described[10]. Monoclonal antibodies against IL-32 (A11C9, YbdYbiotech, 1:200), CD1b (Bcd3.1, ATCC, 1:50), CD68 (EBM11, Dako, 1:100) and NOD2 (2D9, Thermo Scientific, 1:20) were used.

Additional methods. Detailed methodology is described in the **Supplementary Methods**.

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